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The objective of this proposal is to develop new methods of early breast cancer detection by identifying increases in angiogenic growth factor secretion in nipple aspirate fluid (NAF). Specifically, the study is examining FGF-2 (basic fibroblast growth factor) and VEGF (vascular endothelial growth factor), two of the most potent angiogenic molecules whose expression is thought to increase as an early event in breast carcinogenesis. By comparing levels of these growth factors in NAF samples from women from three groups, i.e. those with normal breasts, DCIS (ductal carcinoma in situ), and early invasive breast cancer, we will determine whether increases in either of these molecules heralds the transition to the pre-invasive and/or invasive phenotype.

This report covers primarily patient accrual activities during the second year of the project. These activities include finalizing the clinical protocol for identifying, enrolling, and obtaining samples from women in the three groups, and implementing regular enrollment of subjects, and initial analysis of samples.

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FOREWORD

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Bruce Trock 10/24/00

PI – Signature Date

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INTRODUCTION

The objective of this proposal is to develop new methods of early breast cancer detection by identifying increases in angiogenic growth factor secretion in nipple aspirate fluid (NAF). Specifically, the study is examining FGF-2 (basic fibroblast growth factor) and VEGF (vascular endothelial growth factor), two of the most potent angiogenic molecules whose expression is thought to increase as an early event in breast carcinogenesis. By comparing levels of these growth factors in NAF samples from women from three groups, i.e. those with normal breasts, DCIS (ductal carcinoma in situ), and early invasive breast cancer, we will determine whether increases in either of these molecules heralds the transition to the pre-invasive and/or invasive phenotype.

This report covers primarily patient accrual activities during the second year of the project. These activities include finalizing the clinical protocol for identifying, enrolling, and obtaining samples from women in the three groups, and implementing regular enrollment of subjects, and initial analysis of samples.

BODY

Progress in the study during the second year of funding will be described below with respect to each of the tasks in the original Statement of Work (only those tasks expected to begin during Year 2 will be described).

Task 1: Coordinate with physicians, nurses and scheduling secretaries to receive schedules of patients who will undergo breast surgery (Surgery Clinic), and patients who will attend the Comprehensive Breast Center (CBC), or the Breast Cancer Consultation Group (BCCG).

Progress is greatly improved over last year. To date, samples have been obtained from 128 women. We have determined that the most efficient way to obtain samples is to have the Research Assistant concentrate her time in two surgery clinics, one in Georgetown, and the other of a former Georgetown surgeon who is now in private practice. The reason is that accrual of patients with breast cancer prior to surgery is the most difficult part of the protocol. Patients at this time are often in an understandable state of high concern, and may have too many more important decisions to make than participation in a research study. We have found that the best approach is to enroll women at the time of biopsy, whenever possible. Women are less distracted at this time and more willing to participate. However, the majority will turn out not to have breast cancer. Thus, we also obtain controls from the surgery clinics. In addition, controls are still enrolled in the CBC, as in the previous year. Because women who have undergone breast biopsy with negative results are at increased breast cancer risk (Gail model), these women are not a "normal risk" control group. However, they are still a group of great interest. Therefore, we enroll all women who go to biopsy from the surgery clinic, women who have had their biopsy diagnosis but no additional treatment, or women who will not be biopsied, as follows:

Normal Risk Controls: Women who come for regular exam in the breast surgery clinic, or in the CBC, with no excess risk factors.

High Risk Controls: Women with negative biopsy at the time of enrollment (or previously), or high risk due to family history.

Atypical Ductal Hyperplasia (ADH): Women with biopsy-proven ADH as most advanced lesion.

Carcinoma in-situ (CIS): Women with biopsy-proven ductal or lobular CIS as most advanced lesion.

Invasive Breast Cancer (IBC): Women with biopsy-proven IBC.

Task 3: Implement patient accrual and NAF collection (Months 2-30).

As described above for Task 1, accrual has greatly increased over last year. We are currently enrolling 2-4 patients per week from the surgery clinics and, until recently, the CBC. There has been a temporary halt in accruals from the CBC. The nurse practitioner who enrolled patients for us has left the university, and an active recruitment is underway for her successor. However, because of our success in enrolling both high risk and normal risk controls from the surgery clinics, we don't anticipate that this temporary situation will compromise our overall recruitment goals.

Task 4: Conduct ELISA assays for VEGF and FGF-2 (Months 4-30).

Dr. Sandra McLeskey, the Co-PI who was overseeing the assays in her lab, has left to join another university. Dr. Dorraya El-Ashry, a breast cancer basic scientist with peer-reviewed funding, has taken over this role in her lab. She recently performed VEGF analyses on 48 samples from 33 patients (for 15 patients, samples from both breasts were analyzed). In addition she analysed samples of breast cyst fluid from an additional 11 patients. These patients were included to provide us with a wider range of breast abnormalities that may influence composition of breast secretions. We are still obtaining pathology reports from these patients, so we don't have the analyses stratified by pathology status. The data are attached to this report as an Appendix.

CONCLUSION

Study efforts in this second year of the project have focussed on patient accrual. The obstacles of the previous year have been overcome and accrual is proceeding at a good pace from two surgical clinics (patient populations of 3 surgeons) and one breast health clinic (Comprehensive Breast Center). In addition to normal risk controls our accrual protocol has enabled us to accrue a significant number of women at increased risk for breast cancer. We experienced some delay in beginning the assays of VEGF and FGF-2 due to the Co-PI's leaving the university, to be replaced by Dr. Dorraya El-Ashry and her laboratory. Her lab recently completed initial assays of NAF for VEGF, and will shortly begin assaying for FGF-2. Retrieval of pathology report data is a high priority for the coming year. It is difficult to obtain this data from patient charts, as the charts are not always readily available in the clinic, either because they are being used by a physician, or they have been returned to the Medical Records department. For this reason, we are working with the Surgical Pathology department to obtain photocopies of pathology reports as soon as they are available. This should permit more rapid assignment of a patient to one of the study categories, and allow statistical comparison of VEGF and FGF-2 data to begin.

We are currently enrolling 2-4 patients per week, and anticipate no trouble completing accrual in the final year of the study.

APPENDICES

1. Data from initial analyses of VEGF in NAF and Breast Cyst Fluid (pps. 10-11).
2. List of abbreviations and acronyms (p. 11).
3. Meeting abstracts during reporting period (p. 12).
4. Publications during reporting period (p. 12).
5. Manuscripts in preparation (p. 12).
6. Personnel receiving pay from this negotiated effort (p. 12).

Initial Analysis Results of VEGF in NAF or Breast Cyst Fluid

SAMPLE ID	CYST OR NAF	BREAST SIDE (L, R)	VEGF (pg/ml)
CF001	CYST		7788
CF002	CYST		16862
CF003	CYST		5424
CF004	CYST		29056
MP109	NAF	L	43802
MP109	NAF	R	26662
TS034	NAF	L	79786
MP112	NAF	L	287.8
MP113	NAF	R	15380
MP113	NAF	L	14404
CF007	CYST	L	8700
CF007	CYST	R	10024
MP119	NAF	R	0
MP119	NAF	L	0
MP120	NAF	L	139498
MP120	NAF	R	119488
MP118	NAF	L	26000
MP118	NAF	R	73202
C248	NAF		18564
C251	NAF		0
MP122	NAF	R	23006
TS037	NAF	R	634.4
TS037	NAF	L	5392
CF005	CYST		16768
MP110	NAF	L	0
MP111	NAF	R	43451
MP111	NAF	L	26588
MP112	NAF	L	5603
MP112	NAF	R	1511
CF006	CYST	L	0
MP123	NAF		8970
MP125	NAF	R	3862
MP125	NAF	L	9928
MP128	NAF	R	756.4
	NAF		350.1
CZ204	NAF	R	8912
CF008	CYST	R	1002
CF009	CYST		11437
CB206	NAF	R	9638
CF010	CYST	L	15036
CF011	CYST		20580
CK210	NAF	R	12192

SAMPLE ID	CYST OR NAF	BREAST SIDE (L, R)	VEGF (pg/ml)
CE211	NAF	L	34192
CM212	NAF	R	30797
CM212	NAF	L	42116
CK213	NAF	L	0
CK213	NAF	R	19042
CZ204	NAF	L	14949
C250	NAF	R	27023
C250	NAF	L	20986
CR001	NAF		0
TS038	NAF		0
TS039	NAF	L	7501
TS039	NAF	R	44728
553387	NAF	L	8982
CK217	NAF	L	55951
CK217	NAF	R	25553
TS042	NAF	L	46113
TS042	NAF	R	55712
TS040	NAF	L	24909

LIST OF ABBREVIATIONS AND ACRONYMS

NAF	nipple aspirate fluid
FGF-2	basic fibroblast growth factor
VEGF	vascular endothelial growth factor
CBC	Comprehensive Breast Center

Meeting abstracts during reporting period: None in connection with this project

Publications during reporting period: None in connection with this project

Manuscripts in preparation: None in connection with this project

Personnel receiving pay from this negotiated effort:

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